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BubR1-meditated signaling in the regulations of mitotic checkpoint and post-mitotic adapation

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Mitotic checkpoint is a highly conserved mechanism that regulates the cell division and prevents cells with a perturbed spindle assembly from leaving mitosis, thereby improving the fidelity of chromosome segregation. Mitotic checkpoint activation is associated with several checkpoint proteins, including Bub1-3, Mad1-2 and BubR1. Aberrant mitotic cells undergo either the apoptotic cell death triggered by caspase activities or the adaptation with multiploid progeny cells. Thus, apoptosis of these aberrant cells is important for the prevention of chromosomal instability, such as that observed in human cancers. We explored the molecular mechanisms of BubR1, and identified that, in the adapted aneuploid cells, BubR1 was degraded possibly through ubiquitin-proteasome pathway, and the expression of BubR1 strongly induced the apoptotic cell death and the regression of tumor development. In addition, our study shows evidence that alterations of the phosphorylation of BubR1 protein is responsible for abrogation of the mitotic checkpoint and the resulting chromosomal instability. These results provide important clues for elucidating that the chromosomal instability by mitotic defect is correlated with the post-mitotic destabilization and the apoptotic activity of BubR1 protein, and that BubR1 functions as a tumor suppressor to prevent abnormal mitotic cells with chromosomal instability from adapting. Despite the importance of BubR1 mitotic checkpoint kinase in genomic integrity, very little is known in regards to what the target substrates of BubR1 kinase are, how BubR1 is phosphorylated during mitosis, and how BubR1 is involved in the prevention of the adaptation. Thus, a critical task in understanding the molecular basis of the BubR1-mediated signaling which is responsible for the mitotic checkpoint may be defining the genes required for establishing this checkpoint. In our approach to answering these questions, we have adopted the complex-proteomic and yeast two-hybrid assays, and screened novel BubR1-interacting proteins. In this study, we analysed the potential role of novel BubR1-interacting proteins in the regulations of both mitotic checkpoint and post-mitotic adaptation. Throughout the studies described above, the analysis will yield new insights into the molecular workings of a checkpoint mechanism that ensures a high fidelity of chromosome segregation.

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How does RASSF1A control cell cycle progression from G1 to mitosis?

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The tumor suppressor RASSF1A (RAS association domain family protein 1A) is inactivated in many carcinomas and is implicated in regulation of the cell cycle. RASSF1A regulates mitotic progression by inhibiting both the activity of APC-Cdc20 and microtubule dynamics. However, the regulation of RASSF1A action during cell cycle progression has remained unknown. Here, we have characterized the regulation role of RASSF1A at the G1-S transition and during mitotic progression by providing evidence that RASSF1A is phophorylayed by several kinases. First, we found that Aurora A and B sequentially associate with RASSF1A and catalyze its phosphorylation on serine-203 in vivo during mitosis. Nonphosphorylatable S203A mutant of RASSF1A led to a marked delay in mitotic progression as a result of the failure of RASSF1A to dissociate from Cdc20 and the consequent delayed destruction of mitotic cyclins and the defects of cytokinesis. These findings implicate Aurora-mediated phosphorylation of RASSF1A in regulation of both the timing of mitotic progression and cytokinesis. Interestingly, cyclin D-Cdk4 also phosphorylates RASSF1A on serine-203 at the G1-S transition. We further found that Skp2 (an oncogenic subunit of the SCF ubiquitin ligase complex) binds to and ubiquitinates RASSF1A, and promotes its degradation at the G1-S transition phase of the cell cycle and this Skp2-dependent destruction of RASSF1A requires phosphorylation of the latter at serine 203 by cyclin D-Cdk4. Taken together, the phosphorylation of RASSF1A on the same residue by Aurora kinases and cyclin D-Cdk4 contributes to the regulation of both mitotic progression and G1-S transition.

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A novel function for HSF1-induced mitotic exit failure and genomic instability through direct interaction between HSF1 and Cdc20

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Although heat shock factor (HSF) 1 is a known transcriptional factor of heat shock proteins (HSPs), the existence of pathways independent of the induction of HSPs have been speculated. In the present study, the regulatory domain of HSF1 (amino acid sequence 212-380), but not of other HSFs, such as, HSF2 and HSF4, was found to interact directly with the amino acid sequence 106-171 of Cdc20. Moreover, the overexpression of HSF1 inhibited mitotic exit and the degradations of cyclin B1 and securin. HSF1 overexpressing cells produced aneuploidy and multinucleated cells, but regulatory domain deficient HSF1 did not. Moreover, the depletion of HSF1 from cells highly expressing HSF1 reduced heat shock mediated aneuploidy production. In addition, HSF1 overexpressing cells were found to show elevated levels of micronuclei and genomic alteration. The association between HSF1 and Cdc20 inhibited the interaction between Cdc27 (an APC component) and Cdc20, the phosphorylation of Cdc27, and the ubiquitination activity of APC. The depletion of HSF1 from cells highly expressing HSF1 reduced nocodazole mediated aneuploidy production in cells. These findings suggest a novel function of HSF1 to inhibit APC/C activity by interacting with Cdc20, and to result in aneuploidy development and genomic instability.

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ATM/ATR kinases signaling pathways mediate DNA damage-induced activation of endothelial nitric oxide synthase

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Recently it has been reported that nitric oxide (NO) regulated expression of cell cycle-related genes and caused directly intracellular DNA damage. However, the effect of DNA damage on the activity of endothelial nitric oxide synthase (eNOS) is not elucidated. We explored whether DNA damage altered eNOS activation, and also we investigated its underlying molecular mechanism. Ultra violet (UV) irradiation was treated as DNA damaging agent. UV irradiation acutely increased NO production in bovine aortic endothelial cells (BAEC). This increase was accompanied by increased eNOS phosphorylation at serine 1179 (eNOS-Ser1179) without an alteration in eNOS-Thr497 or eNOS-Ser116 phosphorylation. No alteration in amount of eNOS expression was found. Treatment with caffeine, inhibitor of ATM/ATR kinases, inhibited phosphorylation of eNOS-Ser 1179 in the UV-irradiated BAEC. Furthermore, treatment with small interference RNA (siRNA) of ATR completely attenuated UV irradiation-stimulated eNOS-Ser1179 phosphorylation while siRNA of ATM partially reduced its phosphorylation, suggesting an involvement of ATM/ATR kinases. Other DNA damaging agents, hydroxyurea and aphidicolin, also showed the same effects on the involvement of ATM/ATR kinases in eNOS phosphorylation. In conclusion, our results indicate that DNA damage activates eNOS through the ATM/ATR kinases-mediated eNOS-Ser1179 phosphorylation.

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Differential role of Akt1 and Akt2 in cellular response to DNA damage

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Protein kinase B (PKB)/Akt has been implicated in tumorigenesis through promotes cell proliferation and inhibits apoptosis. Three isoforms of Akt family, termed Akt1/PKBα, Akt2/PKBβ and Akt3/PKBγ, are encoded by three separated genes with more than 86% sequence homology and share similar protein structure. Although recent studies reported that Akt/PKB isoform proteins play unique role in vivo, the isotype-specific role of Akt family protein in DNA damage response has not been addressed. In this study, the isotype-specific function of Akt in cellular survival upon DNA damage was examined using previously generated isogenic wild type, Akt1-/- and Akt2-/- mouse embryonic fibroblasts (MEFs). Both Akt1-/- and Akt2-/- MEFs showed similar increase of sensitivity to γ-irradiation (IR) compared to the wild type MEFs. However, Akt2-/- MEFs exhibited marked hypersensitivity to UV irradiation than wild type and Akt1-/- MEFs. Akt2-/- mouse aortic endothelial cells (AECs) also showed similar hypersensitivity to UV irradiation compared to the wild type and Akt1-/- MEFs. The sharp increase of the sub-G1 phase, the cleavage of pro-caspase-3 and PARP, and induction of DNA ladder formation upon UV irradiation indicate that UV-induced cell death of Akt2-/- MEFs due to apoptosis. Finally, it has been shown that the ectopic overexpression of Akt2 increased the resistance to UV-induced cell death. Taken together, these results suggest that Akt isoforms play different role in cellular response to DNA damage and Akt2 is important to cellular survival in response to UV irradiation.

S9-11

AIMP3 Haploinsufficiency inhibit p53 activation by oncogene including Ras and c-myc, result to Genomic instability

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Generally, oncogenic signals try to lead cells to transformation. But rather, by various tumor suressing mechanism, cell could be guided to other state such as death and senescence. p53 is representative tumor suppressor. And AIMP3 up-regulate p53 in response to DNA damage. Here we suggest AIM3 link oncognic signals such as Ras and c-myc to p53 activation. When expression of AIMP3 was insufficient, various oncogenic signal could not induce p53, increasing the susceptibility of transformation. And the transformed AIMP3+/- MEFs showed chromosomal abnormality in dividing stages. Thus, AIMP3 plays important roles in activating p53 via several oncogenic stresses and regulating the stability of chromosomes.